Experimental and modelling approach to plant-*Rhizobium* interaction

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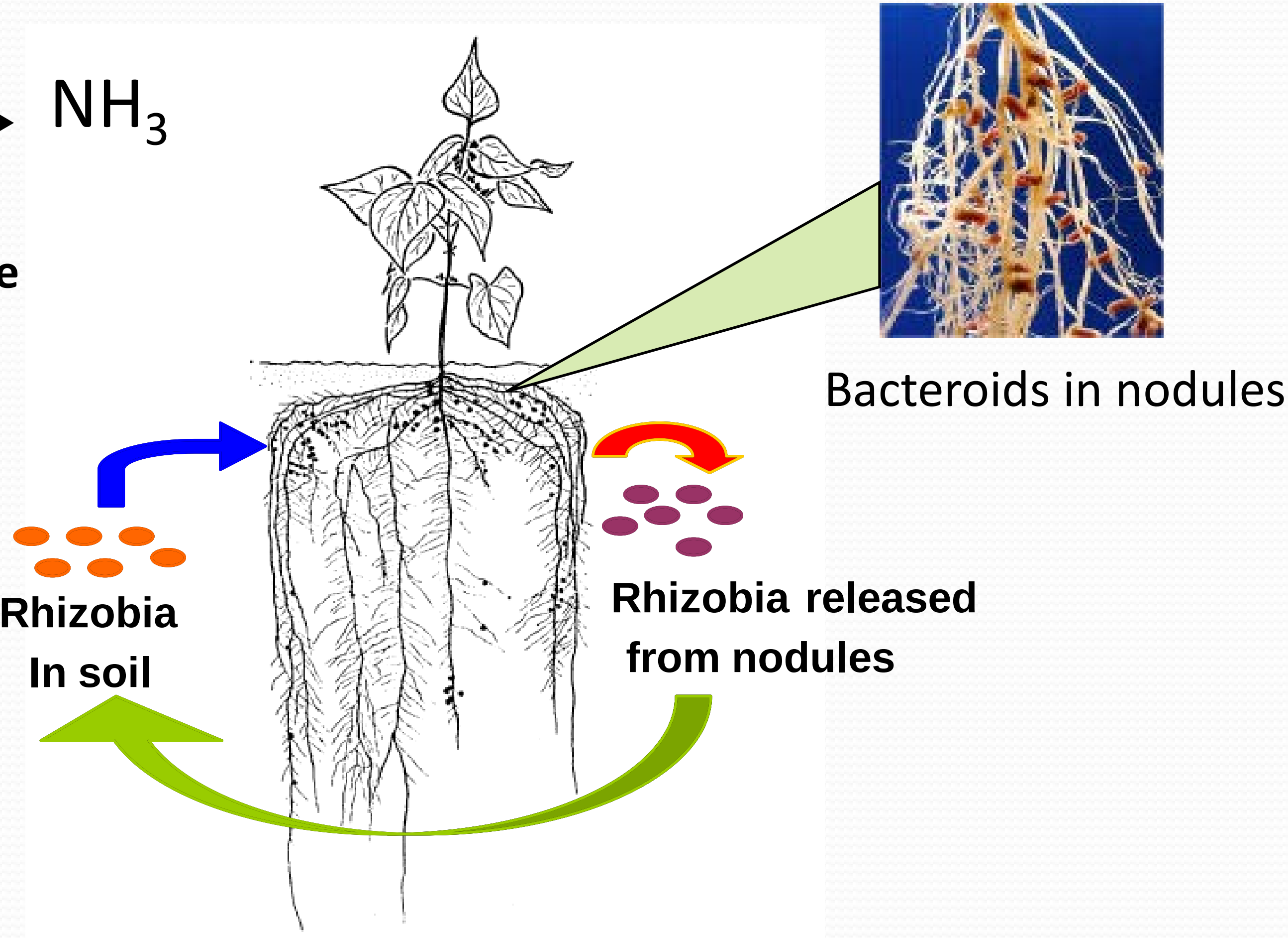
The *Rhizobium*-legume mutualism

$N_2 \longrightarrow NH_3$

Bacteroid nitrogenase



In *Rhizobium*-legume mutualism, partner benefits are clear:

- Plant receives nitrogen from bacteroids inside the nodules
- Bacteroids receive carbon compounds from the plant

But strains of nodulating rhizobia that do not fix (or fix low) nitrogen are common in the field

- Nodulation by ineffective rhizobia is an example of “cheating” by a partner mutualist
- Cheating (receiving benefits but not reciprocating) is widespread in cooperative systems and it has been found in some mutualisms

So, how can cooperation be maintained if partners seek only self-benefit?

- Different mechanisms of sanctioning or rewarding partners have been proposed

In *Rhizobium*-legume mutualism a **plant sanction hypothesis** has been proposed:

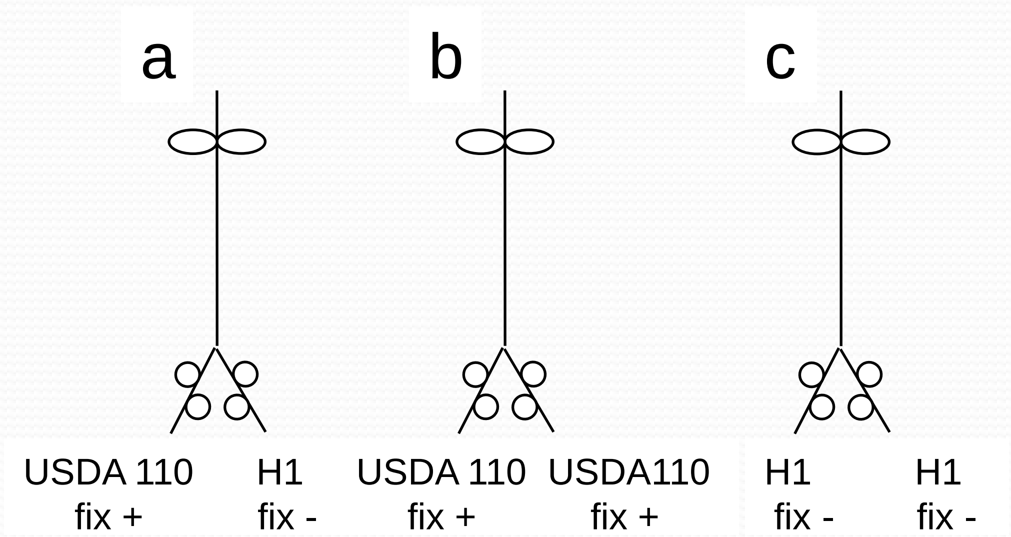
- The plant would sanction ineffective rhizobia by reducing their survival and/or by accelerating nodule senescence (Denison 2000)
- Kiers et al. (2003) tested the hypothesis by preventing N_2 fixation growing nodules in an Ar: O_2 atmosphere, and found decreased survival of bacteroids
- This was proposed as evidence of plant sanction
- They proposed a reduction of O_2 permeability in nodules as the mechanism behind the sanction

However…

…the Ar: O2 causes itself a reduction in nodular O_2 permeability (Hunt & Lyzell 1993).

So, a different approach using split-root soybean plants and two rhizobial strains, one effective (fix+) and its mutant, ineffective isogenic derivative (fix-) was used (Marco et al. 2009a,b; Marco et al. 2013):

- ✓ The fix- strain is a “perfect” cheater, since it has the nitrogenase gene mutated but it shows the same fix+ competitive and nodulating abilities
- ✓ Half-roots of the same soybean plant were independently inoculated with either the fix+ or the fix- strain:


a
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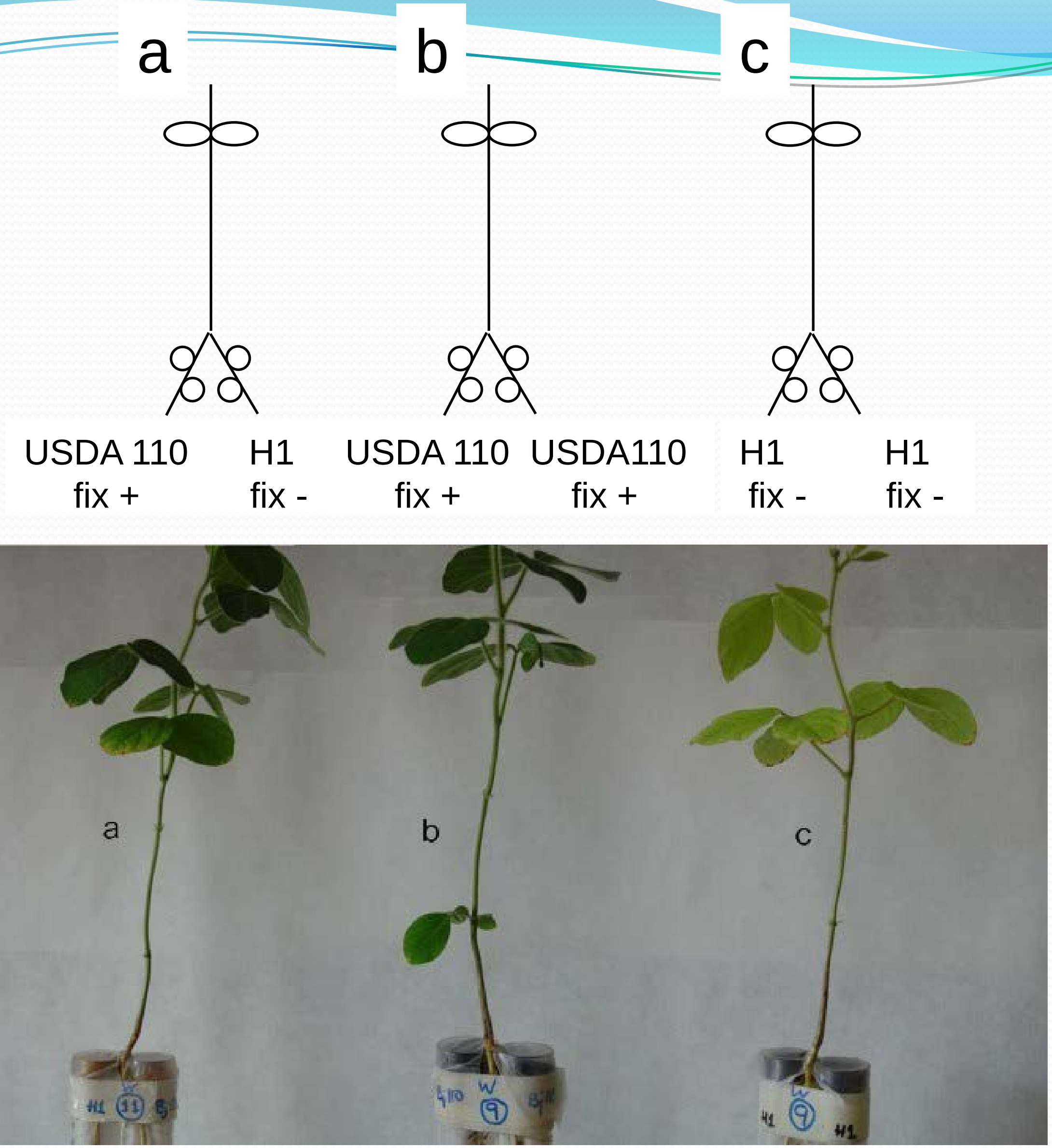
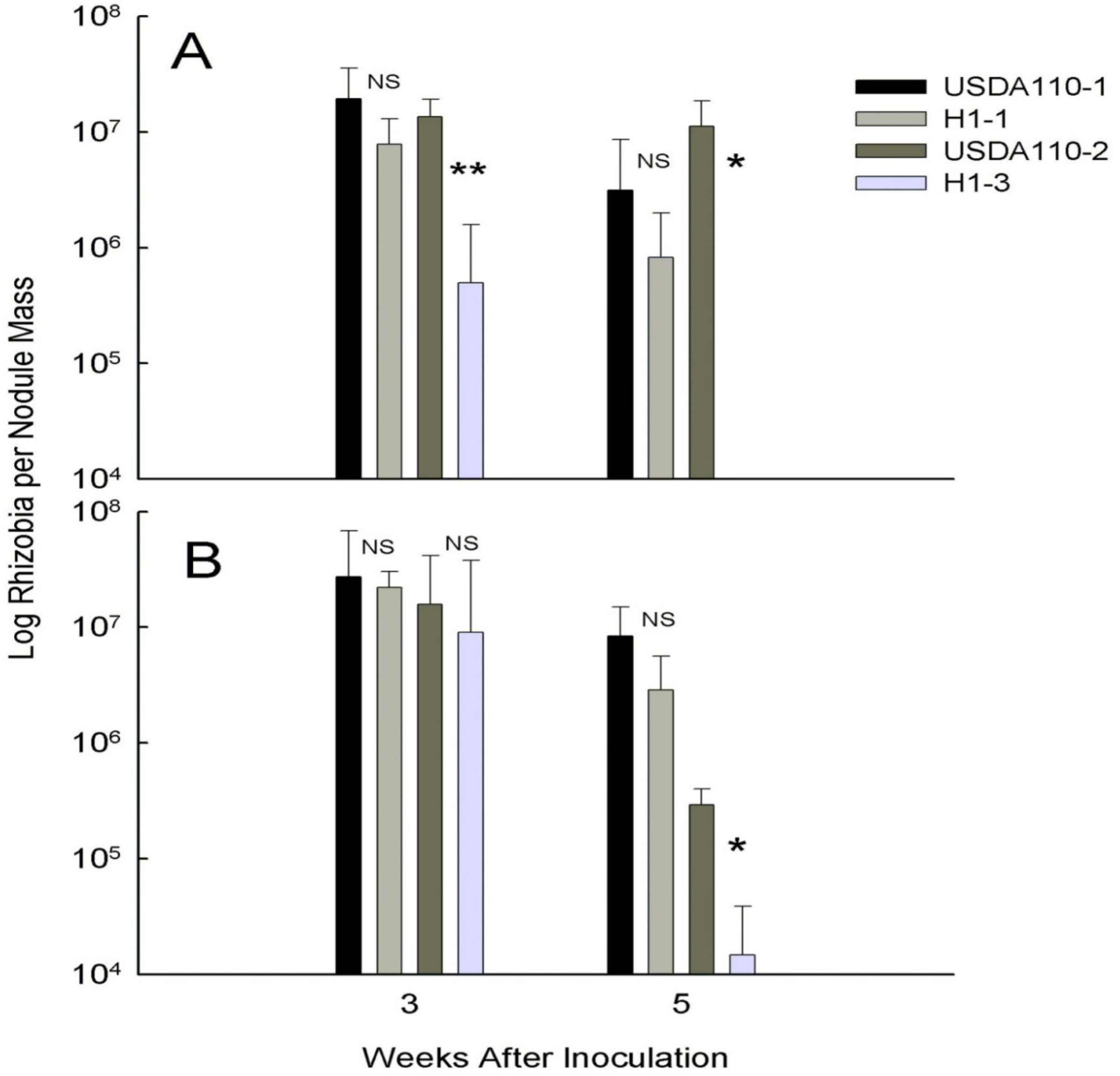


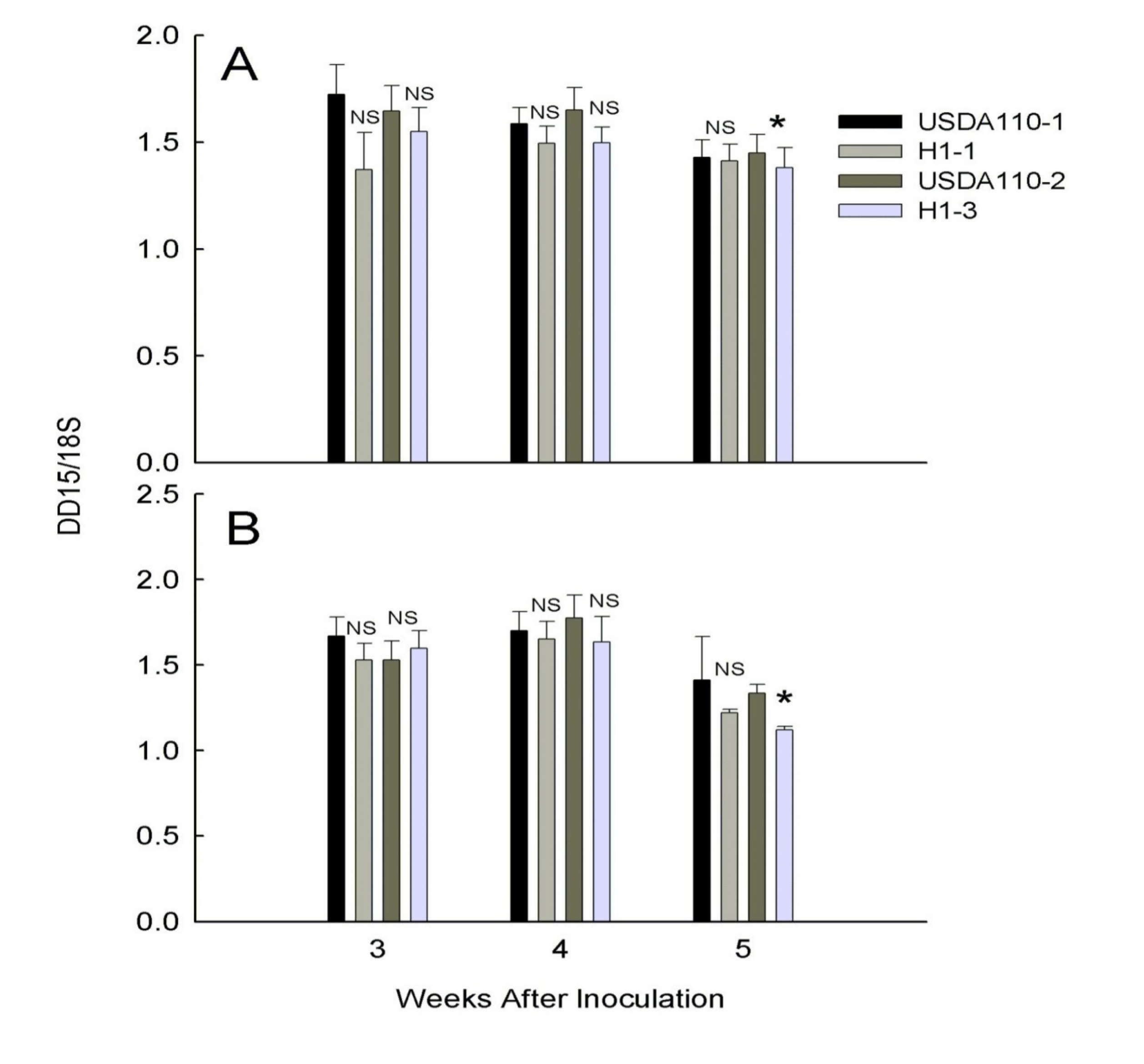
Split-root plants after 4 weeks of rhizobia inoculation

- Bacteroid survival from nodules was determined
- Nodule senescence was determined using a specific molecular marker

No evidence of plant sanction was found:

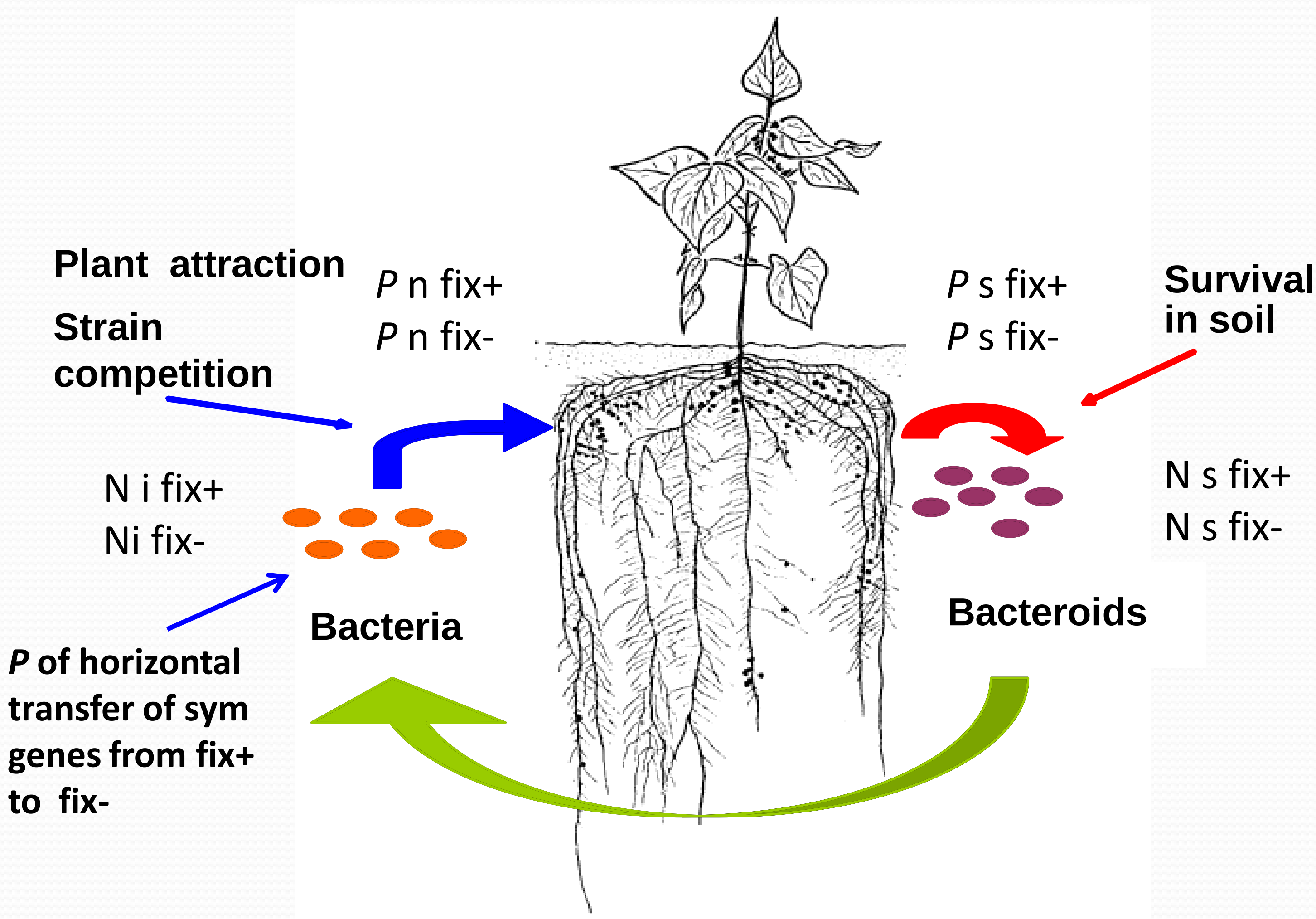


Marco et al. 2009. Acta Oecologica 35: 664-667.



Marco et al. 2009. Acta Oecologica 35: 664-667.

How to model the *Rhizobium*-legume mutualism?

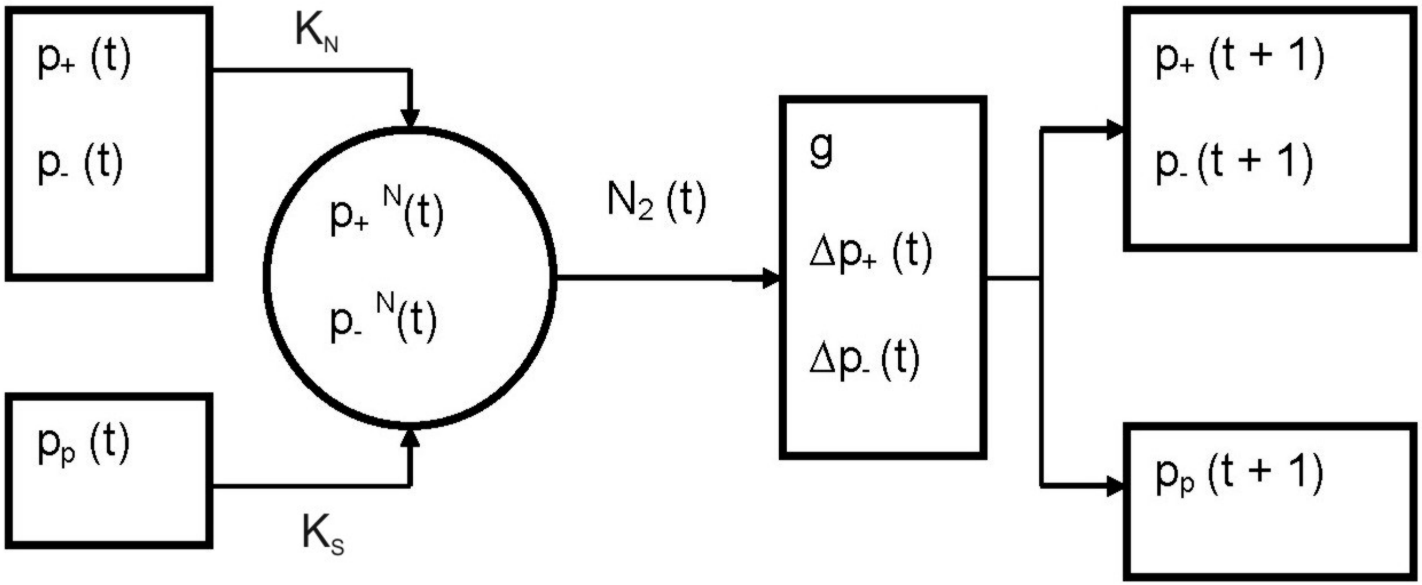


Model assumptions

- Nodules are occupied by either a fixing or a non-fixing strain.
- Nodules are functionally equivalent and metabolically independent of each other.
- Fixing and non-fixing nodules can coexist in the same plant.
- Plants cannot discriminate between fixing and non-fixing rhizobia prior to infection, but
- they can discriminate between fixing and non-fixing nodules.
- A minimum bacterial population is required for nodulation.
- A minimum number of nodules colonized by fixing bacteria is required by the plant to produce seeds, and seed production is proportional to fixed N_2 (up to a maximum).

The mutualistic plant–rhizobia system is described using three logistic maps:

- One map represents the plant population
- The other two account for the populations of free fixing and non-fixing bacteria living in the soil closely surrounding the root.



p+, p-: fix+, fix- rhizobial populations ; p_p: plant population; N: nodules; g: seed production; K_N : total number of nodules; K_s: total root sites available for nodule initiation; N_2: fixed N_2

Marco et al. 2009. J. Theor. Biology 259: 423-433

- Fixing and non-fixing bacterial populations in soil:

$$p_i(t+1) = \left(p_i(t) + \Delta p_i(t)\right)\left[1 + r_i^{\,s}\left(1 - \frac{P_T(t)}{\delta_s}\right)\right] \qquad i = +,-$$

- Total bacterial density in soil:

$$P_T(t) = p_-(t) + \Delta p_-(t) + p_+(t) + \Delta p_+^{\,N}(t)$$

- Plant population density:

$$P_p(t+1) = \delta_p\left[1 - \exp\left(-g(t)\frac{\left|\ln\left(1 - P_g\right)\right| P_p(t)}{\delta_p}\right)\right]$$

- Soil bacterial and plant populations are coupled by K_s

To incorporate plant sanction (σ):

- Without sanction, the number of fix+ and fix- rhizobia released to the soil is the same ($\Delta p+ = \Delta p-$),

  $\sigma = 0$ (rhizobia from all fix- nodules released to the soil)

- With sanction ($\Delta p+ > \Delta p-$) :

  Moderate sanction, $\sigma = 0.5$ (rhizobia from half of fix- nodules released)

  Total sanction, $\sigma = 1$ (no fix- nodule releases rhizobia)

Parameter	Value	Description
r_s	$10^{-4}\ g^{-1}$	Intrinsic rate of growth of bacteria in the rhizosphere (per g of soil)
δ_s	$10^{6}\ g^{-1}$	Rhizosphere´s carrying capacity (per g of soil)
δ_n	10^{6}	Nodule´s carrying capacity
δ_p	$2 \times 10^{5}\ Ha^{-1}$	Plants´ field carrying capacity
m_s	$1.5 \times 10^{5}\ g \cdot Ha^{-1}$	Soil mass per hectare associated to plant population
n	45	Typical number of nodules per plant
K_0	$0.15 \times n$	Minimum number of fixing nodules per plant needed for seed production
G	55	Maximum number of viable seeds produced per plant
P_g	0.69	Probability of a viable seed reaching the adult stage
σ	0-1	Sanction intensity 0 = No sanction, 1 = maximum sanction
p_m	$0 - 10^{3}\ g^{-1}$	Minimum bacteria population per g of soil needed to trigger the nodulation process

Some model results:

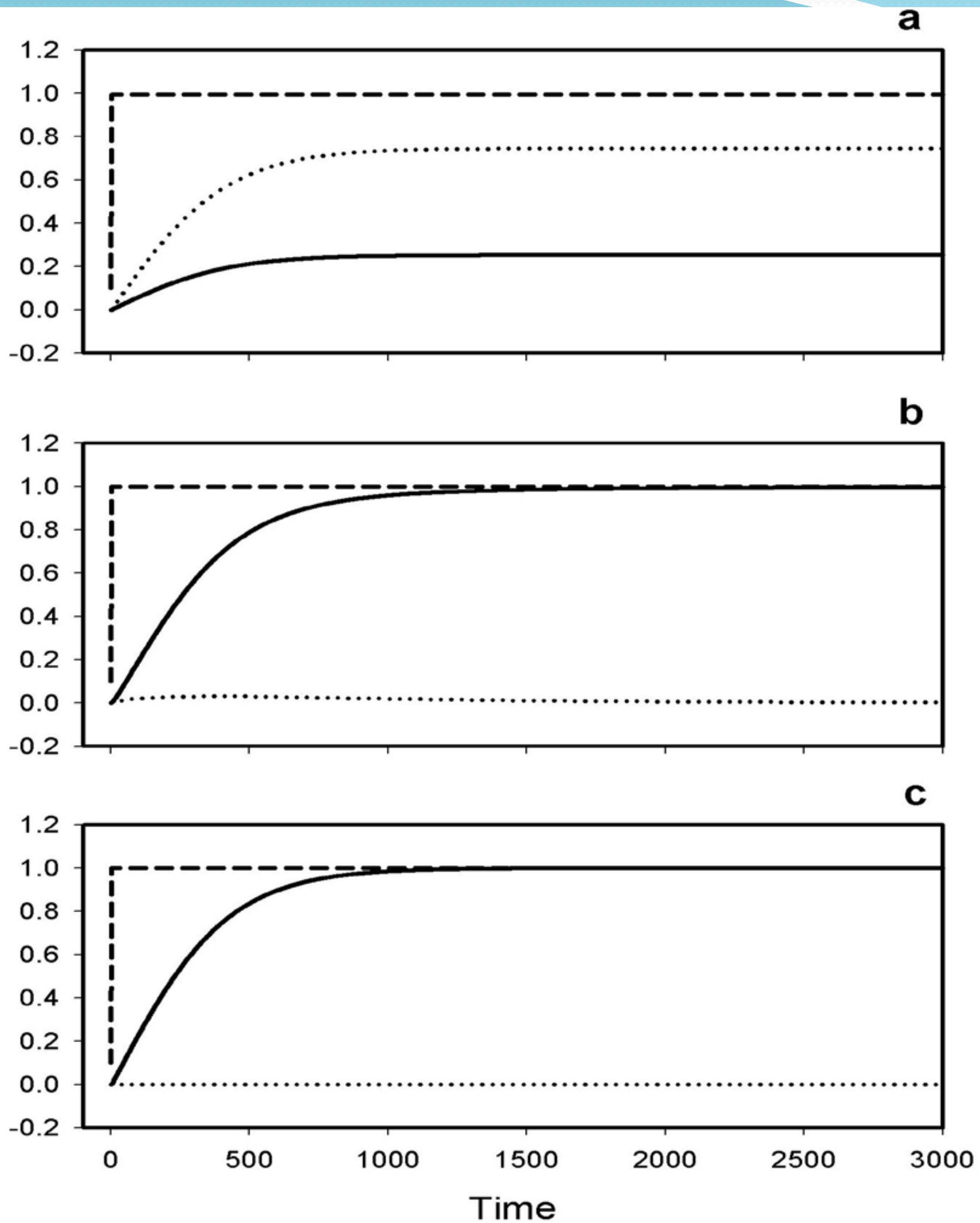
- Simulation results (at population level) are in agreement with experimental results (at individual plant level). Coexistence of fixing and non-fixing (cheating) rhizobial strains is possible under realistic conditions without including plant sanctions.

- Inclusion of plant sanctions leads to the unrealistic result of fix- strains dissapearing from soil.

-----Pp

— P_+

...... P-



No sanction,

$\sigma = 0$

Moderate sanction,

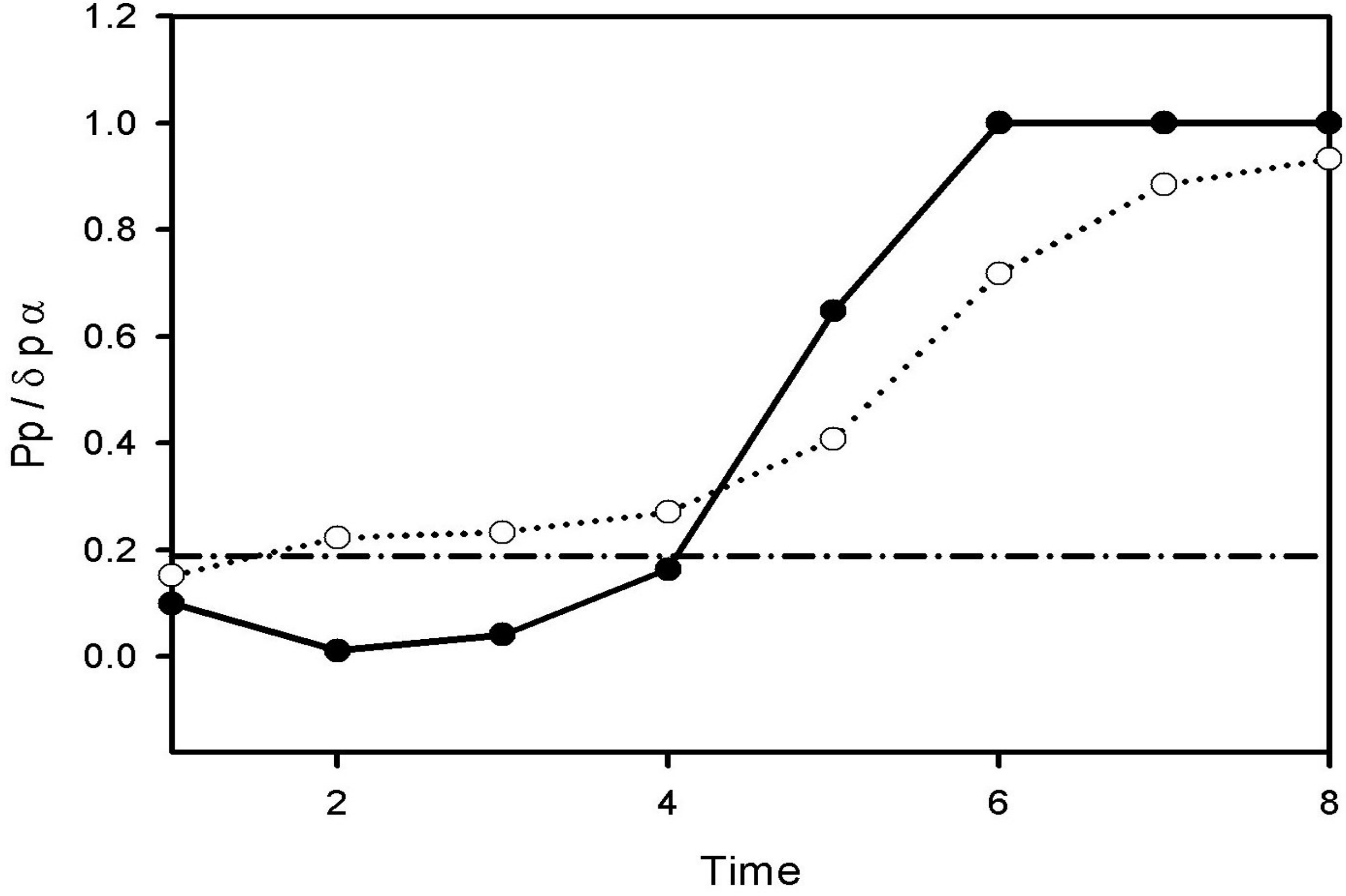
$\sigma = 0.5$

Total sanction,

$\sigma = 1$

- The model predicts a critical fraction of fixing rhizobia in soil (α_c), represented by the fixing
 rhizobia needed to provide a minimum nitrogen amount for plant population persistence (field evidence from Parker et al. 2006)

- With sanction, a small reduction in α_c was found, but only at unrealistically low values of $p_+ < 1 \times 10^3$ bacteria/g^{-1} of soil, smaller than the number of rhizobia needed to support a crop (5.8 x 104 bacteria/g^{-1} of soil (Thies et al., 1995) and to trigger nodulation



Final plant population (fraction of carrying capacity δp) depends on **α**.
Pp/δp (filled circles), **α** (empty circles). $\boldsymbol{\alpha_c}$ is the dotted horizontal
line. When α exceeds the critical value the plant population starts increasing.

Conclusions

- ✓ Coexistence between fixing and non-fixing strains can be explained with our model without sanctions. Inclusion of sanction in the model leads to the unrealistic result of non-fixing strains disappearing from the soil.
- ✓ Simulated plants are able of maintaining viable populations despite being cheated by non-fixing rhizobia when they can get some amount of fixed N_2 from the fixing rhizobia occupying some nodules.
- ✓ Results from modelling (population level) are consistent with experimental results (plant level). Experimental and modelling approaches can provide complementary evidence to test hypotheses in mutualistic systems.

Going further…

We are working further on our model to include:

- Competition between fixing and non-fixing rhizobia in the soil (Hirsch, 1996) and/or for nodulation (Amarger, 1981).
- Co-occupation of the same nodule by strains with different fixation abilities (Rolfe and Gresshoff, 1980). This would counteract plant sanctions (Marco et al., 2013).
- Horizontal transmission of symbiotic genes, turning non-nodulating strains into nodulating rhizobia (Sullivan et al., 1995). The effect would depend on nodulation and/or fixation abilities transferred.

Collaborators

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Dr Juan Sanjuán (Plant-Microorganism Interactions Lab)
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